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VARIATION AND INHERITANCE IN SIZE IN TRYPANOSOMA LEWISI[1]

1. LIFE-CYCLE IN THE RAT AND A STUDY OF SIZE AND VARIATION IN "PURE LINE" INFECTIONS[2]

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The flagellate, Trypanosoma lewisi, is a non-pathogenic blood parasite occurring in various species of rats all over the world. It is known to be transmitted from rat to rat by the rat flea. This trypanosome was selected for the present work because it occurs in the latitude of Baltimore and the vertebrate and invertebrate hosts can easily be reared in the laboratory.

The general plan of the present work on size in T. lewisi is to make a careful study of size and variability in a pure line and then with this background to attempt to explain the facts observed in infections occurring in nature. After a pure line infection was obtained the following questions were attacked: (1) What are the mean and the coefficient of variation? (2) Does growing the same "pure line" in different vertebrate hosts cause significant differences in the mean size or in the coefficient of variation? (3) Does passage of the "pure line" through the invertebrate host cause significant differences in the mean or coefficient of variation? This question gives us a chance to test the possibility that passage of the "pure line" through the invertebrate host may cause a splitting up of the "pure line" into heritably diverse lines. After a study is made of these questions we can attack the final one: (4) Does an infection occurring in nature consist of a large number of "pure lines" such as has

been described by Jennings[8] and others in free-living protozoa which differ among themselves but are *per se* constant in size? As will be seen later, these questions cannot be approached with any degree of exactness until a thorough study is made of the changes in mean size and variability throughout the course of an infection. The present paper deals with these changes in size and variability and with the first of the questions enumerated above. The other three questions are to be taken up in a later report.

A study of inheritance in a parasitic protozoon such as T. lewisi is of interest from several points of view. In the first place the results are of interest from a comparative standpoint when considered in the light of recent advances in our knowledge of the genetics of free-living species. In the second place, the results may be of value in the interpretation of the results of the many studies on the production of strains of parasitic organisms which exhibit new characteristics. Finally the work is the first of a program of investigations, the ultimate object of which is a study of the mechanism of the formation of new lines exhibiting such characters as arsenic-fastness and the inheritance of these characters after passage through both the vertebrate and the invertebrate hosts.

While lack of space prevents a discussion of technique in detail, it may be noted that every precaution was taken to use microscopical technique such that the trypanosomes would be free from shrinkage and distortion. All measurements were made from camera lucida outlines drawn at a magnification of ×3000. The unit used in measuring the drawings was 3 mm.; consequently all of the determinations given in this paper are in actual microns. In making the determinations 100 specimens, taken at random without selection, were drawn in each case. In isolating single organisms with which to start "pure line" infections, a sensitive mercury pipette was used in conjunction with a Barber pipette holder. Figure 1 is a diagram of a trypanosome indicating the various parts of the organism and the distances measured in this work. The names of the parts of the trypanosome run vertically and the abbreviations of the six distances run horizontally.

Size and Variation throughout a "pure line" Infection.—The infection in the rat can be divided into three periods: (1) the incubation period lasts from 1–7 days and is the time which elapses between inoculation and the first appearance of the trypanosomes in the blood. (2) The multiplication period starts with the first appearance of the trypanosomes in the blood and lasts for 10–25 days. This period is characterized by the great variations in size due to the growth and the multiplication of the trypanosomes. (3) The period of "adult" infection follows the second period and is characterized by the absence of all growth and multiplication. This period lasts from one to many weeks at the end of which time the trypanosomes disappear from the blood and the rat is

immune to another infection with T. lewisi. It is apparent that in an organism which shows a cycle of growth and division such as characterizes T. lewisi, we must make all comparisons of size and variability at the same stage in the cycle, and that if there is a period in which there is no growth and division comparisons should be made during this period.

A study of the changes in the means and in the coefficients of variation of the different parts of the trypanosome throughout the course of a "pure line" infection demonstrates very clearly that there are different periods of the infection and indicates the stage at which to make comparisons of size and variability between different infections. Let us take, for example, the coefficients of variation and the means for total length in rats 116 and 105 which are shown in figure 2. The infection in rat 116 was started from a single trypanosome and rat 105 was inoculated from rat 116. In other words, although the infection in the first rat started from a single specimen and in the second rat from many specimens, all of the trypanosomes in both rats are descendants of the single organism injected into rat 116. We can make no determinations during the incubation period since no one knows where the organisms are at this time. Let us consider first the constants for rat 105. On the first day of the blood infection the mean length was $24.785 \pm .423$. This rose rapidly until the 5th day when it reached $30.108 \pm .280$. This rise continued gradually until it reached $31.412 \pm .065$ by the 19th day. From the 19th until the 32nd day, at which time the infection disappeared from the blood, there was no significant change in the mean. The coefficient of variation behaved in much the same manner as the mean. On the first day of the infection it was $26.52 \pm 1.35\%$. It dropped very rapidly for the first seven days and then much slower for the next twelve days. By the 19th day it reached the low value of $3.11 \pm .14\%$ and it showed no significant change from this value throughout the remainder of the infection.

It is well to compare the conditions found in rat 116 with those in rat 105. Rat 116 is given here because a longer time elapsed before the infection reached the adult stage than was the case in any of the other infections studied. This is due probably to the fact stated above, viz., that the infection in rat 116 started from a single trypanosome while the others although they were "pure lines" were sub-inoculated from 116, and in consequence were started with a large number of specimens. As these curves are probably expressions of the resistance of the host to the parasite, we would expect that this resistance would increase more rapidly when the infection is started with a large number of trypanosomes than when it is started with only one. We cannot compare the shape of the curves in the two rats very well because there are not enough points in the curve for rat 116. One thing is probably true, however, and that is that both the mean and the coefficient of variation reach a constant

value by the 25th day. At first it looks as if the mean tends to rise even until the 32nd day. That this is not the case, however, is shown by the fact that the value on the 25th day does not vary appreciably from that of the 72nd day.

The same type of result was obtained for all of the six distances shown in figure 1, namely, for the distances posterior end *to* parabasal body, parabasal body *to* nucleus, nucleus *to* anterior end, anterior end *to* end of

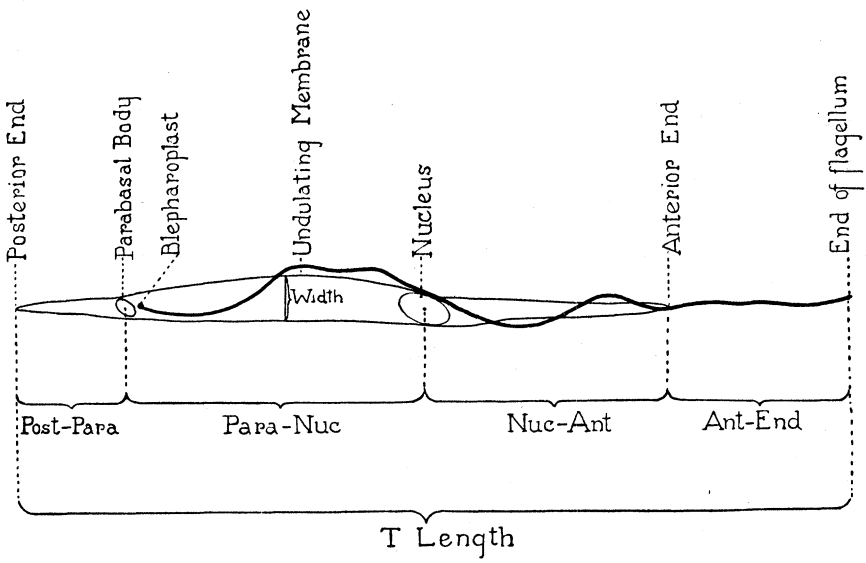

FIG. 1

Diagram of T. lewisi showing the parts of the organism and the distances measured in this work. The parts of the organism are placed vertically while the distances are placed horizontally. The latter consist of the distance from (1) posterior end to parabasal body, (2) parabasal body to nucleus, (3) nucleus to anterior end, (4) anterior end to end of flagellum, (5) total length, and (6) width.

flagellum, and width, although most of the constants do not reach as low a level as is the case with those for total length (see table 1). Curves of this nature were obtained in four rats. These results prove, what we had been led to believe from cytological evidence, viz., that there is practically no division or growth in T. lewisi after the 25th day of the blood infection.

After it was determined that there was no significant change in the mean or coefficient of variation after the 25th day we decided to make all measurements after this day. Most of the measurements which will be given in a later report were made on the 30th day of the blood infection. The fact that the trypanosomes in the blood of the rat reach what we can consider an adult stage makes size a very favorable character with

which to work. It affectively eliminates growth factors from our computations.

The most interesting feature in the study of the variability exhibited by the "pure line" is that once the organism has reached the adult stage of infection the coefficients of variation are extremely low. Table 1 shows the constants for rat 105. The coefficients of variation for total length and parabasal-nucleus are 2.80% and 2.21%, respectively. It is to be noted also that these two characters show a lower coefficient of variation than any of the others. After considering this subject in great detail we have come to the conclusion that these two characters give the truest index of the variability in the "pure line." While there is not

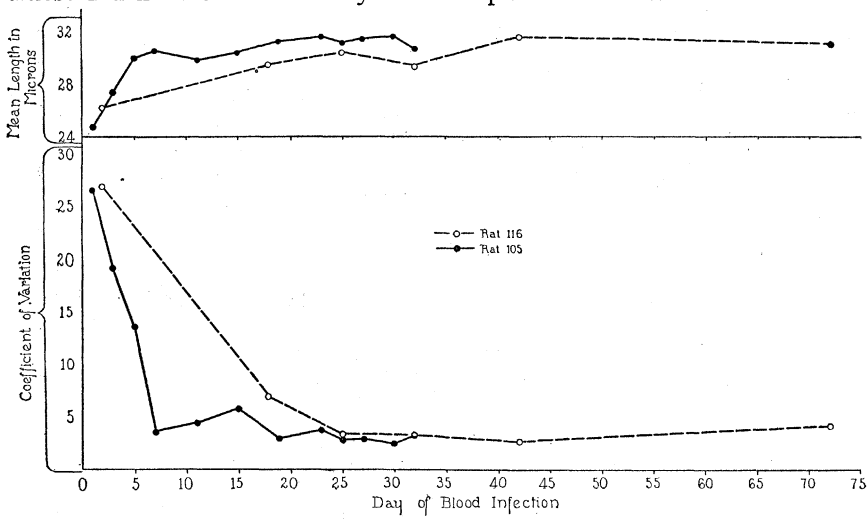

FIG. 2

Graph showing the changes in the mean and coefficient of variation for total length in rats 116 and 105. The infection in 116 was started from a single organism and 105 was inoculated from 116. The trypanosomes disappeared from the blood of rat 105 on the 32nd day and from 116 on the 72nd day.

much doubt that posterior-parabasal is more variable than either of these two, we feel that much of its variability as well as that of the other distances with high coefficients is due to the difficulty of making the measurements.

Conclusion.—We can draw the following conclusions from this part of the work: Trypanosoma lewisi reaches an "adult" stage in its development in the rat in about 25 days after it appears in the blood. Once this stage is reached there is practically no division or growth. Due to the elimination of growth factors, the organisms show a very low coefficient of variation in "pure line" infections, provided they are measured after the "adult" stage is reached. These facts make T. lewisi a very favorable organism in which to study size and variation.

TABLE 1

CONSTANTS FOR THE SIX DISTANCES GIVEN IN FIGURE I FOR THE "PURE LINE" IN RAT 105 ON THE 25TH DAY OF BLOOD INFECTION. THE MEANS AND STANDARD DEVIATIONS ARE IN MICRONS

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Post-Para	4.268 ± .036	.544 ± .025	12.74 ± .61
Para-Nuc	10.854 ± .016	.240 ± .011	2.21 ± .10
Nuc-Ant	9.511 ± .047	.704 ± .033	7.41 ± .35
Ant-End	6.619 ± .068	1.010 ± .048	15.27 ± .74
T. Length	31.251 ± .059	.875 ± .041	2.80 ± .13
Width	1.590 ± .015	.230 ± .010	14.47 ± .70

[1] This and a later report form a preliminary account of a series of investigations which are being carried out in this laboratory on variation and inheritance in T. lewisi.

[2] Throughout this work the term "pure line" infection has been used to designate an infection, the trypanosomes of which have all arisen from a single organism. A given "pure line" may either have been started from a single specimen or it may have been sub-inoculated from such an infection.

[3] See especially Jennings, H. S., *Proc. American Phil. Soc.*, **47**, 1908 (393–546). *American Nat.*, **43**, 1909 (321–337). *Ibid.*, **45**, 1911 (79–89).

MEASUREMENT OF THE DIAMETER OF ALPHA-ORIONIS BY THE INTERFEROMETER

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It was shown in these PROCEEDINGS[1] that in the application of interference methods to astronomical measures, the fringes show no decrease in visibility with the slits separated by the full aperture of the 100-inch Hooker telescope even when the seeing is poor. It was therefore decided to build an interferometer with movable outer mirrors in order to test for separations as great as 20 feet.

The interferometer bed consists of a fabricated steel beam, designed with special regard to lightness and stiffness, mounted on the end of the Cassegrain cage of the 100-inch reflector (fig. 1). Two tracks were planed on the top, true to 0.001 inch (0.025 mm.), the frame being supported on the planer as it was to be mounted on the telescope. On this beam are mounted four slides, each carrying a mirror about 6 inches (152 mm.) in diameter, inclined 45 degrees to the base. The two inner mirrors M_2, M_3, are fixed, 45 inches (114.2 cm.) apart, while those of the outer pair M_1, M_4, are movable and can be separated to a distance of 20 feet (6.1 m.). The light pencils are reflected from the outer to the inner mirrors, thence over the customary path a, b, c, d, in the telescope, and are viewed with an eyepiece at the Cassegrain focus d, where the equivalent focal length is 134